

1.0 PURPOSE

The purpose of this procedure is to establish a protocol for the uniform use of the Nikon ECLIPSE 55i microscope.

2.0 SCOPE

This standard operating procedure (SOP) is applicable to the caHUB Pathology Resource Center (PRC) pathologists and Comprehensive Data Resource (CDR) personnel.

3.0 RESPONSIBILITY

- 3.1 The PRC pathologists will be responsible for examining representative tissues on H&E stained microscope slides and occasionally on specially stained slides. The results are qualitative data consisting of confirmation of the tissue or diagnosis initially provided by the BSS, quantitative data consisting of measurements of the optical image made by the PRC pathologist, as well as additional annotations of areas of interest.
- 3.2 The CDR will be responsible for maintaining the quantitative data and digital photographs.
- 3.3 caHUB will retain the glass microscope slides.

4.0 DEFINITIONS

- 4.1 BSS - Biospecimen Source Site
- 4.2 CAPT – caHUB Clinical and Anatomic Pathology Team
- 4.3 CBR – Comprehensive Biospecimen Resource
- 4.4 CDR - Comprehensive Data Resource
- 4.5 Digitally scanned image – a digital image of a whole microscope slide tissue section routinely stained with hematoxylin and eosin or other designated stain
- 4.6 DS-L2 camera – digital camera connected to microscope
- 4.7 H&E – hematoxylin and eosin
- 4.8 Nikon ECLIPSE 55i – dual headed microscope
- 4.9 PA - Formalin-fixed, paraffin-embedded (FFPE) tissue
- 4.10 PRC – caHUB Pathology Resource Center

5.0 ENVIRONMENTAL HEALTH & SAFETY

N/A

6.0 MATERIALS/EQUIPMENT

- 6.1 Nikon ECLIPSE 55i – dual headed microscope with camera (See 9.0 ATTACHMENTS)
- 6.2 Nikon DS camera control unit DS-L2
- 6.3 USB Memory Drive

7.0 PROCEDURE

7.1 Microscope operations.

- 7.1.1 The microscope is switched on by a power switch on the left side (facing the microscope); brightness is controlled by a circular switch on the right side. Photomicrographs should be made with maximum brightness. Field diaphragm and aperture diaphragm are in their customary below stage positions. Interpupillary distance and diopter adjustments are made on the microscope head and oculars.

7.2 Photography and Annotation

- 7.2.1 Turn on camera by pressing power switch on the right lower side of the camera control unit's display stand. The microscope's field image will appear within a minute. Focus the camera image on screen using the microscope's focus knob and then adjust the oculars to match focus.
- 7.2.2 A photograph of the monitor's field is taken by pressing the 'Capture' button on the front of the monitor. Focusing is done from the image on the monitor, not from the microscope's ocular.
 - 7.2.2.1 The background can be whitened by placing the slide to show a clear (section-free) area. Right click the monitor until a small screen appears in the lower right field of the monitor. Click the 'WB' box to whiten the background. A graduated box to the left of the WB can lighten or darken the image without affecting the background.
- 7.2.3 A USB memory stick on the right side of the monitor records the photographic image. The image is automatically identified by its time and date of capture. The microscope's arrow will appear on the photograph; the mouse's arrow will not appear.
- 7.2.4 Displaying microscope's menus. A right click of the camera's mouse on the left side of the monitor displays a menu from which annotations may be made. Do not alter adjustments.
- 7.2.5 Annotating images. Select "TOOL" screen for an on-screen scale, grid, pen, etc. Select 'm1' to 'm5' to correspond to the objective in use, e.g., m1 for 2x objective, m2 for 4x, m5 for 40x. The corresponding scale will appear. Select 'T. Bar' for a ruler or area tool. The m1 to m5 selection also determines the appropriate linear and area measurements. Additional details are in the operating manual (see reference 8.2).

- 7.2.5.1 Annotation. Activate the 'TOOL' screen for annotations. The 'Clear all' icon on the screen allows you to delete an annotation layer (Note that micrometers (um) are changed to millimeters (mm) by moving the decimal point 3 spaces to the left; sq um are changed to sq mm by moving decimal 6 spaces to the left.)
- 7.2.5.2 Scale. A mm or um scale can be activated by selecting the Scale button on the TOOL screen.
- 7.2.5.3 Linear measurements. Activate the 'Ruler tool' icon from the 'T. Bar' and click at one point in the specimen, then at a distance, click again; the length of the line will appear in mm or um.
- 7.2.5.4 Area measurement can be carried out by selecting the area tool (cross hatched) from the 'T. Bar'; outline the area with multiple connecting linear measurements; when the lines are connected, a right click will display the value of the area in squared mm or um.
- 7.2.5.5 Annotation color can be selected from the colored box in the 'T. bar'.
- 7.2.5.6 Deletions of annotations done with the T.bar can be made by selecting the curved arrow on the T. bar. Select 'Clear' on the Tool menu to erase all annotations.
- 7.2.5.7 Photograph tissue section before and after annotating as needed.
- 7.2.6 Completed caHUB tissue reporting form and, as needed, photographs are transmitted to the CDR.
- 7.3 Microscope Maintenance
 - 7.3.1 Microscope should be covered after use with the dust cover. If needed, Lens Cleaner can be used to clean oculars and objective lenses.
 - 7.4.2 Microscope Calibration. This is carried out by the Nikon technician whose address is by the microscope.

8.0 REFERENCES: Instruction manuals

- 8.1 Nikon ECLIPSE 55i – dual headed microscope
- 8.2 Nikon DS camera control unit DS-L2

9.0 ATTACHMENTS



Nikon ECLIPSE 55i microscope.



Camera and control unit's display stand (monitor).



NIKON MICROSCOPE EX.XXXX Operation and Maintenance Procedure

PR-9001

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APPROVALS

NAME / TITLE	SIGNATURE	DATE

INITIATION/REVISION HISTORY

REV #	DESCRIPTION OF CHANGE	AUTHOR	EFFECTIVE DATE